DEVELOPMENT AND EVALUATION OF NANOCARRIER BASED NANOEMULSION OF MEDICINAL PLANTS FOR THE TREATMENT OF URINARY TRACT INFECTION

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by

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DECLARATION

I hereby declare that the work presented in this report entitled **"DEVELOPMENT AND EVALUATION OF NANOCARRIER BASED NANOEMULSION OF MEDICINAL PLANTS FOR THE TREATMENT OF URINARY TRACT INFECTION"**, will be carried out by me. I have given due credit to the original authors/sources for all the words, ideas, diagrams, graphics, computer programs, experiments, results, that are not my original contribution. I have used quotation marks to identify verbatim sentences and given credit to the original authors/sources.

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CERTIFICATE

Certified that **MUKESH KUMAR** has carried out the research work presented in this synopsis entitled **"DEVELOPMENT AND EVALUATION OF NANOCARRIER BASED NANOEMULSION OF MEDICINAL PLANTS FOR THE TREATMENT OF URINARY TRACT INFECTION"** for the award of **Doctor of Philosophy (Pharmaceutical Sciences)** from IIMT College of Medical Sciences, IIMT University, Ganganagar, Meerut, under the supervision of **Dr. Divya Pathak (Associate Professor)**. The synopsis embodies results of original work, and studies are carried out by the student herself and the contents of the synopsis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Signature
Dr. Divya Pathak
(Associate Professor)

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CHAPTER - 1

The goal of enhancing therapeutic benefit while avoiding toxicity is achieved through an optimum medication delivery system. As time has gone on and science and technology have advanced, dosage forms have changed from straightforward mixtures and pills to extremely complex systems called as innovative drug delivery systems. Nano emulsions are one example of an innovative drug delivery technology.

1.1 Nanoemulsion

Oil-in-water dispersions known as Nano emulsions are composed of oil, water, and Smix (a mixture of surfactant and co-surfactant), which has a size range of 1 to 200 nm. These are biphasic systems in which tiny droplets of one phase are dispersed into the other phase. **[1].** The size of the nanoemulsion, which is a biphasic dispersion with an oil phase, an aqueous phase, and a higher proportion of surfactant and co-surfactant mixture, ranges from 50 to 200 nm. These systems have intimately scattered phases that transition into one another. There are two different kinds of nano emulsions, including bicontinuous and oil/water (o/w) or water/oil (w/o) **[2].** Whereas the outer phase is referred to as the external phase or continuous phase, the dispersed phase is also known as the internal phase or discontinuous phase. These two varieties of nanoemulsion include bi continuous and oil in water (o/w) or water in oil (w/o) types. They seem transparent because of the narrow size range **[3].** It can also be said that nano emulsions are stabilised by an interfacial coating known as a surfactant molecule, are isotropically transparent dispersions, and are thermodynamically stable. Moreover, it goes by the names mini, submicron, and ultrafine emulsions **[4].** Because of their small droplet sizes, nanoemulsions are stable and seem transparent and translucent, as seen in figure 1.1. **[5]**. The usage of nanoemulsion in cosmetics and medicine delivery **[6].** and because to its small droplet size, superior solubilization powers, and long-term stability **[7]**. The main distinction between emulsion and nanoemulsion is that the former is thermodynamically unstable while the latter is kinetically stable. While emulsions appear hazy, nanoemulsions are physically quite transparent. **[4].**

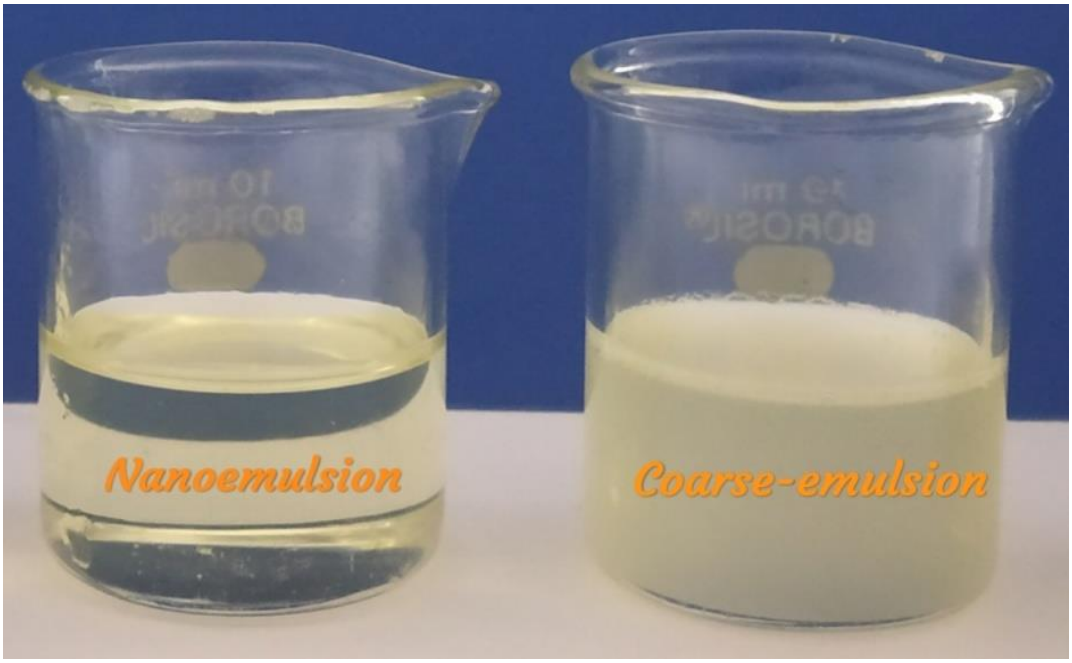

Figure 1.1: Nano emulsion v/s Coarse-emulsion

1.1.1 Advantages of nanoemulsion

1. It offers site-specific medication delivery **[8].**
2. It increases the drug's bioavailability.
3. It has increased the drug's physical stability.
4. It makes it easier for the medicine to dissolve in its lipophilic carrier. **[9]**.
5. Minimal energy/work is added during nanoemulsion preparation.
6. A nanoemulsion has a high amount of hydrophobics that it can dissolve.
7. It makes a great medicine delivery system since it prevents drugs from degrading and maintains their stability throughout time. **[10].**
8. This could be utilised as an alternative to liposomes and vesicles.
9. It is non-toxic and non-irritating.
10. It offers better absorption due to the smaller droplet size and larger surface area of the droplets **[11].**
11. Several formulations, such as creams, liquids, foams, and sprays, are possible **[12].**
12. This is also utilised to disguise tastes.
13. It improves the technology for cell culture's uptake of oil-soluble nutrients.
14. Greater dissolving efficiency are offered by nanoemulsion **[13].**

1.1.2. Nanoemulsion's drawbacks

1. GIT lipase partially hydrolyzes and digests surfactant molecules.
2. Excessive usage of co-surfactants and surfactants in excessive amounts slowed its functionality **[14]**.

1.2 Composition of Nanoemulsion

1.2.1 Oil phase

oil's physicochemical characteristics, such as its viscosity and molecular volume. Significantly influence the spontaneousness of the nano-emulsification process, nanoemulsion droplet size, typical drug solubility, and the oil that has the maximum drug solubilizing property for the formulation of nanoemulsion **[15]**.

The chosen oil must be capable of producing nanoemulsion with tiny droplet sizes. Due to its propensity to solubilize hydrophobic or lipophilic drugs, oil plays a crucial role in nanoemulsions. This helps the medication to self-emulsify and moreover aids in the drug's intranasal delivery. Oil's ability to form emulsions is strongly correlated with its molecular make-up. The chosen oil needs to be able to create nanoemulsions with tiny droplet sizes **[16]**.

Example of a common oil phase in a nanoemulsion **[17]**

1. **Fixed oils-** Soybean oil and castor oil
2. **Medium chain triglyceride-** Triglycerides of capric/caprylic acids
3. **Medium chain mono and di-glycerides-** Mono and di-glycerides of capric or caprylic acids
4. **Long chain monoglycerides-** Glyceryl monooleate
5. **Propylene glycol fatty-acid esters-** PG monocaprylate, PG monolaurate/dilaurate

The drug's solubilization determines the choice of the oil phase, which can create the necessary nanoemulsion properties. The size of the nanoemulsion is directly related to the drug's lipophilicity and the percentage of oil in the nanoemulsion. In some circumstances, a blend of oils can be employed to achieve the best oil phase qualities. Both nanoemulsion and microemulsion have been used using a similar principle.

1.2.2 Surfactant

For the creation of a nanoemulsion, the choice of surfactant is also essential. The process of nano-emulsification and the size of the droplets are significantly influenced by surfactant qualities such as the needed HLB value for the oil phase, its viscosity, and its affinity towards the oil phase. The nanoemulsion's droplet size is significantly influenced by the surfactant concentration in it. The chosen surfactant system's regulatory requirements and its suitability for the specific route of administration must also be taken into consideration. Many non-ionic surfactants, such polyethylene glycol (PEG), have the propensity to modify intestinal permeability and medication uptake in relation to P-glycoprotein driven efflux. However, this surfactant may also have negative effects that vary on the concentration, the structure, and the mode of administration. At greater concentrations, certain surfactants may induce skin irritation and gastro intestinal inflammation. For the creation of nanoemulsions, a number of surfactants are available that can be used singly or in combination to produce nanoemulsions with desired properties while avoiding surfactant side effects **[18]**.

Example of a common surfactant in a nanoemulsion **[19].**

1. **Polysorbates**- Polyoxyethylene-20-sorbitan monooleate
2. **Sorbitan esters**- Sorbitan monooleate, Sorbitan monolaurate, Sorbitan monostearate

1.2.3 Co-Surfactant

Surfactant and co-surfactant selection is dependent on how well they mix with the chosen oil. For miscibility studies, a variety of surfactants and co-surfactants are evaluated **[21].**

Co-surfactant is working in the nanoemulsion to bring down the interfacial tension caused by oil fraction. They can be used in nanoemulsion for a variety of functions, such as:

- Dosage of the drugs capacity increase in nanoemulsion;
- To alter the nanoemulsion's droplet size

Co-surfactants are pharmaceutically acceptable solubilizers that are hydrophilic, lipophilic, or amphiphilic, generally employed. Amphiphilic solubilizers, like propylene glycol (PG), polyethylene glycol (PEG) and glycol ethers (diethylene glycol mono ethyl ether or Transutol P),

are frequently incorporated in nanoemulsion design to increase the Drug loading and the length of time needed for self-nano emulsion **[20]**. Commonly used co-surfactants example. **[19]**

1. Alkane diols and triols, including glycerol and propylene glycol
2. Polyethylene glycol 400 is a kind of polyethylene glycol.

1.3 Oral Administration of drug loaded nanoemulsion

The simplest, most practical, and most economical method of administering non-invasive medications is orally. **[22]** Due to increased patient compliance with tailored treatment, this strategy makes it simple to achieve therapeutic goals **[23].** Patients with paediatric, gastrointestinal, and possibly trauma or epileptic diseases may experience some limitations as a result of their inability to cooperate. Alternately, some medicines with specific physicochemical qualities might not be suitable for a given route of administration. Medication stability in the gastrointestinal tract is seriously hampered by medications with poor water solubility. Yet, some medications, such as peptide medicines, are known to go through enzymatic and hydrolytic breakdown. **[24].** They can only partially penetrate the membrane, and their hygroscopic nature may also be problematic **[25].** There are too many methods for enhancing their bioavailability, some of which include complexation with cyclodextrins, solid dispersion, micronization, and the use of particle delivery systems that are soluble or dispersable in water. **[26].** Using novel drug delivery systems like, microemulsion, nanoemulsion and nanoparticles can help in eliminating certain pharmaceutical challenges like poor bioavailability of drug, irritation caused at nasal site and toxicity upon long-term usage **[27]**. These are all methods to prevent a drug's breakdown in the gastrointestinal tract, direct it to a particular place, and extend the drug's transit time to support absorption pathways.

Wet milling technique is used to preparation of oral nano suspensions for those drugs which are poorly soluble in nature. Based on stirring, oscillating, and turbulent motion, these milling processes for breakdown the drug into the smaller particles for incorporating into the nano suspension for subsequent delivery. Smaller the particle size, could be better able to permeate the GIT membrane. **[28].** For targeting of nanoparticulate (nanoemulsion) drug carriers, oil in water type nanoemulsion was comprised of polyunsaturated fatty acids and will contain Saquinivir, which is promising for HIV/AIDS treatment because it increases the drug's oral bioavailability.

[29]. It has been discovered that HIV or AIDS medications can reduce viral loads below the threshold for detection, which has led to the study of viral reservoir sites. **[30].**

Therefore, through multiple lipid absorption processes, the oils are successfully absorbed in the GIT. Being put into oils is one of the finest ways to increase the absorption of medications, especially protein pharmaceuticals. The use of oils as nanoemulsion loading components is a newly developed method that increases drug absorption in the gastrointestinal tract (GIT).

According to reports, utilising a nanoemulsion in an oral medication delivery system increases the medicine's effectiveness at reaching the target site. The entire investigation of utilising nanoemulsions orally to improve medication bioavailability, permeability, cell and tissue targeting, imaging, and therapeutic effects. **[31].**

Disease:

Every year, 150 million people worldwide are affected by urinary tract infections (UTIs), which are among the most prevalent bacterial illnesses. An estimated 10.5 million office visits for UTI symptoms (representing 0.9% of total ambulatory visits) and 2-3 million emergency room visits were made in the United States alone in 2007. **[36-38]**. The social costs of these infections, including medical expenses and lost workdays, are currently estimated at US$3.5 billion annually in the United States alone. UTIs are a major source of morbidity in women of all ages, elderly men, and young boys. Frequent recurrences, pyelonephritis with sepsis, kidney injury in infants, preterm birth, and difficulties brought on by repeated antimicrobial usage, such as high-level antibiotic resistance and Clostridium difficile colitis, are examples of serious sequelae.

UTIs can be classified as simple or complicated clinically. Those who are otherwise healthy and have no structural or neurological abnormalities of the urinary tract are typically affected by uncomplicated UTIs. **39-40]**, These infections are separated into upper and lower UTIs (cystitis) (pyelonephritis). Female gender, a past UTI, sexual activity, vaginal infection, diabetes, obesity, and genetic predisposition are some of the risk factors linked to cystitis. **[41]**. The term "complicated UTIs" refers to UTIs that are linked to conditions that compromise the urinary tract or the body's natural defence mechanisms, such as urinary obstruction, urinary retention brought on by neurological conditions, immunosuppression, renal failure, renal transplantation, pregnancy, and the presence of foreign bodies like calculi, indwelling catheters, or other drainage devices.

[42-43]. In the US, indwelling catheters are to blame for 70-80% of complex UTIs. **[44]**, involving 1 million cases annually 4. The most frequent cause of subsequent bloodstream infections, catheter-associated UTIs (CAUTIs) are known to increase morbidity and death. Diabetes, feminine gender, older age, and prolonged catheterization are risk factors for acquiring a CAUTI. **[45]**.

Both Gram-negative and Gram-positive bacteria, as well as some fungi, are responsible for UTIs (FIG. 1). Uropathogenic Escherichia coli is the most frequent culprit behind both simple and complex UTIs (UPEC). In terms of prevalence for the agents causing simple UTIs, UPEC is followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. **[46-48]**. (FIG. 1). For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis*, *P. aeruginosa* and GBS9, **[49-50]** (FIG. 1).

Antibiotics are frequently prescribed to patients with symptomatic UTIs; nevertheless, these medications have the potential to permanently alter the normal microbiota of the vagina and gastrointestinal tract and to foster the growth of multidrug-resistant microbes. **[51]**. The possibility of being colonised by multidrug-resistant uropathogens can rise due to the existence of niches that the changed microbiota is no longer able to fill. A crucial point is that the "golden age" of antibiotics is coming to an end, necessitating the development of more effective alternative therapies. In recent research, uropathogens from the urine of women with symptomatic UTIs were directly analysed using RNA sequencing. These studies have been essential in helping us understand the molecular specifics of how uropathogens adhere, colonise, and adapt to the nutritionally restricted bladder environment, evade immune surveillance, and persist and spread in the urinary tract. They also work in conjunction with basic science and improved animal models.

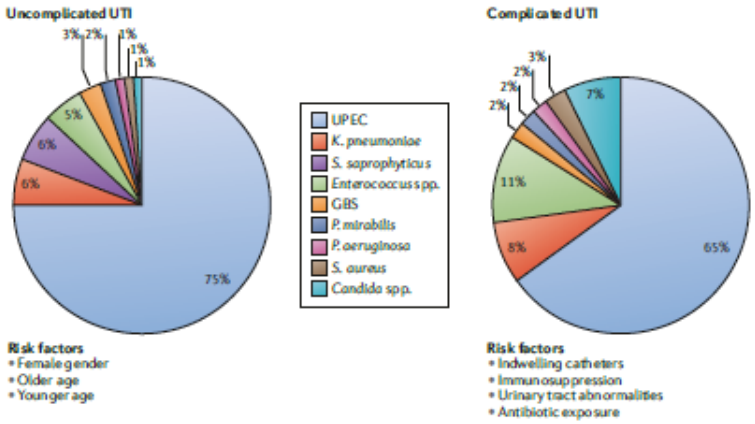

Figure 1: Epidemiology of urinary tract infections.

So, the pathogenic mechanisms that are significant in UTIs can be prevented and combated thanks to the essential virulence factors that have been identified in these investigations. **[52]**. In this Article, we compare and contrast the virulence factors employed by the major uropathogens UPEC, K. pneumoniae, P. mirabilis, E. faecalis, and P. aeruginosa. We also examine the molecular pathways of pathogenesis during bladder and kidney infection. Also, we examine existing antibiotic therapies, mechanisms of antibiotic resistance, novel combination therapies, and upcoming therapeutic strategies that target virulence factors with vaccines and small compounds.

Many pathogens, including fungi and both Gram-positive and Gram-negative bacteria, are responsible for urinary tract infections (UTIs). Women, young children, and elderly individuals who are otherwise healthy are most commonly affected by uncomplicated UTIs. Indwelling catheters, urinary tract abnormalities, immunosuppression, or antibiotic exposure are frequently linked to complicated UTIs. Uropathogenic Escherichia coli is the most frequent culprit behind both simple and complex UTIs (UPEC). Other causal agents for simple UTIs include Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, group B Streptococcus (GBS), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida spp. They are listed in order of prevalence. The other causal agents for complicated UTIs are GBS, Enterococcus spp., K. pneumoniae, Candida spp., S. aureus, P. mirabilis, and K. pneumoniae (in that order of prevalence). **[53].**

2. RATIONAL OF STUDY

The development and assessment of Vaccinium Macrocarpon nanoemulsion for the treatment of bacterial infection in the urinary system is the focus of the current work. Vaccinium Macrocarpon,

especially for women who experience recurrent UTIs, aids in preventing UTIs of the bladder and urethra (the tube that drains urine from the bladder). which is used in place of antibiotics. It is an herbal drug with high solubility, high permeability in intestine and have a very short half-life ranging from **1 to 8 h after dosage**. The juice and topical preparations for UTI treatment is available, but it has been found that, nanoemulsions shows better permeability. The study is also aimed to find which co-surfactant is more effective with the penetration enhancer. Ethanol and PEG400 are the co-surfactants and tween 80 as the surfactant (penetration enhancer).

2.1 ADVANTAGE OF THE RATIONAL

1) Better & viable option for UTI infection.
2) Palatable and appropriately formulated.
3) Fewer side effects.
4) Decreases the cost of hospitalization.
5) Cost effective.
6) More affordable and accessible.
7) Averting the mortalities.

So, the objective of my work is to decrease the frequency of dosage by reducing the bioavailability and half-life of Vaccinium Macrocarpon by preparing it as a nanoemulsion.

CHAPTER – 2

1. **Singh Y. *et al.* (2017);** Review that an ampiphillic surfactant stabilises either water in oil (W/O) or oil in water (O/W) droplets to create a biphasic dispersion known as a nanoemulsion. They appear to be ultrafine dispersions with varied drug loading, viscoelastic properties, and aesthetic characteristics that can be used for a variety of purposes, including drug delivery. [35].
2. **Halnor V.V. *et al*. (2017);** explains that nanoemulsions are transparent, isotropic liquid mixes of oil, water, surfactant, and co-surfactant that are thermodynamically stable. Nanoemulsion typically has droplet sizes between 20 and 200 nm. The size and shape of the particles scattered in the continuous phase is the primary distinction between an emulsion and a nanoemulsion. This technique is intended to overcome a number of issues with traditional drug delivery systems, including limited bioavailability and noncompliance. [36].
3. **Gupta A. *et al*. (2016);** Stated These nanoemulsions are kinetically stable 100 nm or smaller liquid-in-liquid dispersions. Nanoemulsions, which have an optically transparent appearance, are used in a variety of industries, including medicine delivery, food, cosmetics, pharmaceuticals, and material creation. High pressure homogenization, ultra-sonication, phase inversion temperature, and emulsion inversion point, as well as more recently discovered techniques like the bubble bursting method, are utilised to prepare nanoemulsion. [38].
4. **Ozturk B. *et al.* (2015);** Using a model of the mouth, stomach, and small intestine, simulated gastrointestinal tract (GIT) was used to study nanoemulsions made with a natural surfactant (quillaja saponin). These are the decreases in the rate of free fatty acid release during lipid digestion: Mineral oil > fish oil > orange oil > medium chain triglycerides (MCT) > corn oil. Vitamin D3's measured bioaccessibility, on the other hand, declined in the order of: Fish oil is followed by orange oil, mineral oil, MCT, and corn oil. These findings demonstrate that the kind of carrier oil significantly affects vitamin bioavailability and fat digestion. The best method for boosting vitamin bioavailability was to create nanoemulsions using long-chain triglycerides (fish or corn oil). [37].

5. **Jaiswal M. *et al.* (2014);** To address the significant issues with traditional drug delivery systems, a cutting-edge method of drug delivery has been created. This review provides a thorough understanding of a nanoemulsion system. Nanoemulsions are created in order to enhance the distribution of active medicinal components. [13].
6. **Ravi Theaj Prakash U. *et al.* (2011);** This review focuses on the many drug delivery methods using drug-loaded nanoemulsions, highlighting the adaptability of these delivery systems for therapeutic and other relevant applications. [36].
7. **Abdelbary G *et al.* (2008);** claimed that Gentamicin is a member of a group of antibiotic aminoglycosides that have been widely employed in human medicine. Although gentamicin has a significant potential utility against a variety of Gram-negative and Gram-positive bacteria, many of its applications are limited to low dose administrations, mostly due to the development of kidney residues and the expensive nature of the medication. It is used in veterinary medicine and is administered as an injection, cream, ointment, or suspension. Scientists working in the multidisciplinary fields related to the eye, including chemical, biochemical, pharmaceutical, medical, clinical, and toxicological sciences, are interested in the hard challenge of drug delivery in ocular treatments. Antimicrobial medications must currently be repeatedly injected into the infection site to treat endophthalmitis and microbial keratitis, respectively, in order to treat ocular infections. [32].
8. **Kaur IP *et al*. (2004);** This article reviews the limitations of conventional ocular therapy and investigates various novel approaches generally to improve ocular drug bioavailability, the benefits of the vesicular approach over these, and the challenges that will need to be overcome in the future to make the vesicular system more efficient. [33].
9. **Gamazo *et al*., 1999;** said that the necessity to explore the physical targeting of antibiotics by drug delivery systems was motivated by the desire to improve the therapies for microbiological illnesses caused by intracellular bacteria, like Brucella. [34].

CHAPTER - 3

AIM

"DEVELOPMENT AND EVALUATION OF NANOCARRIER BASED NANOEMULSION OF MEDICINAL PLANTS FOR THE TREATMENT OF URINARY TRACT INFECTION"

Objective

1. To perform the formulation studies of *Vaccinium Macrocarpon* Extract with excipients.
2. To formulate the nanoemulsion of *Vaccinium Macrocarpon* using suitable oil, surfactant/co-surfactant.
3. Evaluation of Nanoemulsion formulation based on size, drug content, in-vitro
4. To treat Bacterial Infection in UTI.
5. To enhance Bioavailability by converting it into Nano Formulation.
6. To reduce the adverse drug reaction in relation with *Vaccinium Macrocarpon*.
7. To reduce the dissolution and disintegration time by smaller the particle size.
8. To enhance the absorption of drug across biological membranes.

CHAPTER – 4

Drug Profile:

Common Name: American Cranberry

Other relatives of the cranberry family:

- European cranberry V. oxycoccus;
- lingonberry– V. vitisidaea;
- blueberry– V. myrtillus)

Cranberries are low, creeping shrubs or vines that can grow to a height of 5 to 20 centimetres (2 to 8 in), are up to 2 metres (7 ft) long, and have small, evergreen leaves on slender, wiry stems that are not heavily wooded. The flowers are a deep pink colour with very pronounced reflexed petals that completely reveal the style and stamens and point forward. Bees pollinate them for us. The fruit is a berry that is bigger than the plant's leaves and starts out pale green before turning red as it ripens. It is tasty, although the acidic flavour frequently overpowers the sweetness.

Species and Description:

- ***Vaccinium oxycoccos or Oxycoccus palustris*** (common cranberry, northern cranberry, or cranberry) can be found widely in northern Europe, northern Asia, and northern North America in the chilly temperate Northern Hemisphere. It has tiny leaves with an inrolled edge that are 5–10 mm (1/4–3/8 in) long. On finely haired stems, dark pink blooms with a purple central spike are produced. The fruit has a tart, reviving flavour and is a petite, pale pink to scarlet Raw Cranberry harvest from Kingston, Massachusetts.
- ***Vaccinium microcarpum or Oxycoccus microcarpus*** In northern North America, northern Europe, and northern Asia, you can find (little cranberry). It is quite similar to V. oxycoccos, with the exception of the leaves, which are more triangular and the flower stems, which are hairless. Its stems can also be smaller and may yield less flowers than V. oxycoccos. Although the main distinction is their triangular shapes, they also differ in that their leaves may be smaller in size. Some botanists classify it as a part of V. oxycoccos.
- ***Vaccinium macrocarpon or Oxycoccus macrocarpus*** **(big cranberry, American cranberry, bearberry)** is a high-altitude native of northern North America, spanning

Canada, eastern United States, and south to North Carolina. The bigger, flat, 10–20 mm (3–8–34 in) long leaves and slightly apple-like berry flavour set it apart from V. oxycoccos.

- **Cranberry Fruit Chemical Composition:**

- **Nutritionals:**

The principal nutrients in raw, unsweetened American cranberries are 87% water, 12% carbs, and very small amounts of protein, lipids, and fibre. Citric acid contributes 1.8–2.6% of the 2.1-4.9% titrable acidity in small cranberries. Big cranberries acquire less titrable acidity (1.9–2.4%); citric acid concentrations for the 'Howes' and 'Early Black' cultivars were reported to be 1.88 mg/g and 6.08 mg/g, respectively. The three primary acids that could be identified in the giant cranberry were quinic, malic, and citric. The nutritional makeup of cranberries can vary based on the cultivar, climate, growing circumstances, maturity/ripeness stage, harvesting period, and storage circumstances.

RAW cranberries nutrient content per 100 grammes.

Nutrients	**Values**
H_2O	86.9 g
Energy	45.8 kcal
Carbohydrates	11.9 g
Sugars	4.4 g
Dietary fiber	3.5 g
Fat	0.1 g
Protein	0.4 g
Vitamin B1	0.011 mg
Vitamin B2	0.02 mg
Vitamin B3	0.102 mg
Vitamin B5	0.294 mg
Vitamin B6	0.057 mg
Vitamin B9	1 µg
Ascorbic acid	13 mg
Tocopherol or Alpha-Tocopherol	1.4 mg
Phytonadione	6 µg
Vitamin A	4 µg
Vitamin A, IU	64 IU
Ca	9 mg
Fe	0.24 mg
Mg	5 mg
Mn	0.28 mg

P	12 mg
K	81 mg
Na	3 mg
Zn	0.08 mg
Cu	0.07 mg
Se	0.1 µg

- **Biochemical Constituents:**

  Secondary metabolites found in cranberries include polyphenols, which are chemically varied and have antioxidant and antibacterial activities. **[54, 55].**

  Both the large and the small cranberries are rich in phenolic substances such phenolic acids, flavonoids (anthocyanins and flavonols), and tannins. These chemicals have led to the giant cranberry being acknowledged as a significant dietary and therapeutic agent. Of more than 150 chemicals found in the giant cranberry, flavonoids were the most prevalent.

  Anthocyanins, flavonols, and proanthocyanidins are among the subclasses of flavonoids. The substantial cranberry contained the following:

  26 phenolic acids and benzoates, 13 anthocyanins, and 16 flavonols.

  Anthocyanins, catechins, and flavones are among the flavonoids found in tiny cranberries. Three cranberry cultivars contained 48 polyphenols, including 19 flavonols, 8 anthocyanins, 7 phenolic acids, and 14 flavan-3-ol oligomers.

  Acids Phenolic Hydroxybenzoic and hydroxycinnamic acids, which are present in cranberries, are phenolic acids. High levels of benzoic acid and minor levels of 2,4-dihydroxybenzoic, p-hydroxybenzoic, and o-hydroxybenzoic acids are present in cranberries. P-coumaric, sinapic, caffeic, and ferulic acids are the hydroxycinnamic acids found in cranberries. The cultivar and stage of berry ripening determine the overall amount of phenolic compounds in various cranberry fruit cultivars.

CHAPTER – 5

PLAN OF WORK

Step1: Preformulation studies.

1) Identification of drug on the basis of melting point, UV and FTIR spectroscopy.
2) Drug, oil, and S mix compatibility study by UV/TLC and FTIR.
3) Interaction drug-excipients and excipients-excipients interaction studies.
4) Determination of solubility of drug in solvent, oil, and Smix.
5) Preparation of phase diagram.

Step2: **Optimization and Formulation**

1) Selection of formulation from phase diagram.
2) Formulation methods-
    a) High pressure homogenization
    b) Micro-fluidization
    c) Persuasion method/phase inversion technique
    d) Brute force method

Step 3: Evaluation

Physical Evaluation of formulation.

a) Morphological evaluation droplets size.
b) % Transmittance.
c) Refractive index (RI).
d) Conductivity
e) Viscosity
f) In-vitro permeation studies
g) TEM
h) Microbiological studies
i) In-vitro studies

j) In-vivo Studies

Step 4: Stability studies

- **Accelerated stability studies**

S.N.	**STUDY**	**DURATION**
1	Review of Literature	(Throughout study)
2	Screening and Selection of excipient	2 Month
3	Preformulation study	2 Month
4	Formulation and optimization of dosage form	2 Month
5	Evaluation of dosage form	1 Year
6	Stability study and Thesis writing	6 Month
	Total time period of study	**2 Years**

Future Scope

- With the help of Nanoemulsion improve the bioavailability.
- Increases half life
- The combined application of Cranberry Seed Oil nanoemulsions open a promising avenue for skin conditions.
- enhances their pharmacokinetics and pharmacodynamics via various delivery methods.
- Because the medicine is enclosed in an oil droplet, it is protected against hydrolysis and oxidation.

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